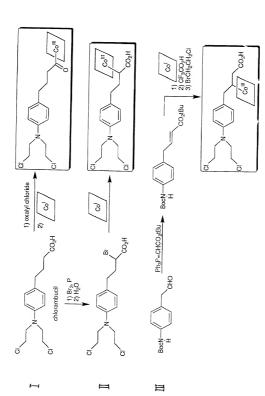
A chlorambucil, ethyl ester-containing bioconjugate can be synthesized by the following methous. When conjugating a drug via a carboxyl group, as in the case of chlorambucil, linking the drug to the cobalamin via a hydroxyethyl tether may be desirable. This can be accomplished by one of two convenient routes, both of which are schematically illustrated below. First, 2-hydroxyethyl-cob(III)alamin can be readily prepared from cob(I)alamin and bromoethanol. Esterification is carried out under standard conditions, i.e. by reaction of a carboxylic acid (chlorambucil) with an alcohol (2-hydroxyethylcob(III)alamin) in the presence of dicyclohexylcarbodiimide (DCC) (or water-soluble derivatives such as EDCI) and a catalytic amount of 4-N,N-dimethylaminopyridine (DMAP) and its hydrochloride salt (DMAP-HCI) in dichloromethane or toluene. Alternatively, the ester-linked conjugate can be prepared by first forming the 2-bromoethyl ester of chlorambucil and then reacting the ester with cob(I)alamin to provide the same product. The reaction schemes (I, II) are shown below. With this mode of attachment, cleavage from the bioconjugate leads to release of the ethyl ester of chlorambucil according to reaction scheme III.



An etoposide-containing bioconjugate can be synthesized by the following method. Etoposide is a semisynthetic derivative of the natural product epipodophyllotoxin that is widely used against a variety of tumors, especially small cell lung carcinoma and germ cell tumors (De Jong et al., 1995). It has also shown considerable promise in the treatment of refractory cases of ovarian and breast cancer. Etoposide appears to function as a topoisomerase II poison.

Etoposide is conjugated to cobalamin, Co[SALEN] and other organocobalt complexes according to the following reaction schemes. Bioconjugates 8a and 8b require conversion of the free phenol of etoposide (3) to the corresponding chloroformate 17. Direct acylation with Co(I) gives acylCo(III) derivative 8a, while treatment with the previously described hydroxyethylCo(III) derivative 18 furnishes carbonate 8b. This derivative is also available via acylation of 3 with the chloroformate 19 derived from 18. Preparation of acetal-modified conjugate 8c may be more challenging. The ethylene acetal of 3 can be hydrolyzed and then the acetal reformed using aldehyde 20a or dimethyl acetal 20b (Keller-Jusl et al., 1971) Compound 20a may also be accessed via careful, selective oxidation of 18, while 20b should be available via alkylation of the Co(I) derivative with commerically available bromoacetaldehyde dimethyl acetal. In addition, the acetal of glucose can be formed and then the secondary alcohol of 21 can be glucosylated. Cleavage of 8a or 8b either give 3 directly via fragmentative pathways, or furnish products which can undergo eventual hydrolysis to 3. Trapping with H• following homolysis of 8c would then furnish 3.